RESPONSES TO PUBLIC COMMENTS ON THE OFFICE OF PESTICIDE PROGRAM'S SCIENCE POLICY

The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorus and Carbamate Pesticides

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CONTENTS

LIST OF ABBREVIATIONS

Scientific Terms:

AChE	Acetylcholinesterase
ADI	Acceptable Daily Intake
BuChE	Butyrylcholinesterease
LOAEL	Lowest-Observed-Adverse-Effect Level
LOEL	Lowest-Observed-Adverse-Effect Level
MF	Modifying factor
NOAEL	No-Observed-Adverse-Effect Level
NOEL	No-Observed-Effect Level
PoD	Point of Departure
RBC	Red Blood Cell (also, erythrocyte)
RfC	Reference Concentration
RfD	Reference Dose
UF	Uncertainty Factor

Organizational Terms:

ACRA	Acute Cholinesterase Risk Assessment Work Group
ACPA	American Crop Protection Association
ARRP	Alliance for Reasonable Regulation of Pesticides
DPR	California Department of Pesticide Regulation
PMRA	Canadian Pest Management Regulatory Agency
FQPA	1996 Food Quality Protection Act
ILSI	International Life Sciences Institute
IPCS	International Programme on Chemical Safety
IWG	FQPA Implementation Working Group
JMPR	FAO/WHO Joint Meeting on Pesticide Residues
NAS	National Academy of Sciences
NRDC	Natural Resources Defence Council
OPP	Office of Pesticide Programs
SAB	Science Advisory Board
SAP	FIFRA Scientific Advisory Panel
TRAC	Tolerance Reassessment Advisory Committee
WHO	World Health Organization

A. INTRODUCTION

In 1997, the U.S. Environmental Protection Agency's (US EPA) Office of Pesticide Programs (OPP) presented a science policy paper on *The Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorus and Carbamate Pesticides* to the FIFRA Scientific Advisory Panel for review and comment (US EPA, 1997). In 1998, as part of its Tolerance Reassessment Advisory Committee (TRAC) review of science policy issues, OPP published the 1997 policy paper for broader public comment (US EPA, 1998). Many persons have commented on this policy (submitted under docket OPP-00480 relative to the 1997 FIFRA Scientific Advisory Panel (SAP) meeting, and under dockets OPP-00557 and OPP-00560 relative to the TRAC process). All of the comments and recommendations have been reviewed by OPP and incorporated into the revised science policy document, as appropriate (US EPA,
2000).

The comments submitted ranged in their specificity. Some commenters addressed the general policy and its rationale as well as all of the specific questions posed, while other reviewers provided detailed comments only on certain aspects of the policy, such as the reliability of regional brain acetylcholinesterase analyses. A listing of the names and affiliations of those who submitted comments is provided at the end of this document.

This response package contains a summary of the most significant revisions to the 1997 Science Policy, followed by responses to comments. These responses are organized around the ten questions originally posed by OPP in its November 5, 1998, request for public comments:

Question 1. How should OPP use measures of cholinesterase inhibition in plasma, red blood cells, and brain in determination of critical effect levels and setting reference doses?

Question 2. *Should plasma and red blood cell measures of cholinesterase inhibition be treated differently from brain measures of acetylcholinesterase inhibition and/or from one another?*

Question 3. *How should measures of [acetylcholinesterase inhibition in] peripheral tissues be used in these processes of risk assessment, both in a practical sense and a science policy sense?*

Question 4. *Can measures of acetylcholinesterase inhibition in peripheral tissues, such as the heart and salivary glands, be used as a supplement or even an alternative to blood measures?*

Question 5. *Should comparative data on cholinesterase inhibition in the young exposed pre-natally, during infancy (nursing), and during childhood be considered essential for defining the relative sensitivity of the young and adults?*

Question 6. *Are other measures, such as functional measures of clinical signs, or learning and memory, similarly important?*

Question 7. *Should EPA require the differentiation of acetylcholinesterase and butyrylcholinesterase in plasma, and how might this data be used?*

Question 8. *Should EPA require receptor binding assays for long term (subchronic and chronic) studies, and how should such data be interpreted?*

Question 9. OPP has proposed to use a weight-of-the-evidence approach that obligates the risk assessor to consider all of the study results as a whole, rather than focusing on any single result in isolation of the others. Is this approach a reasonable means for evaluating the overall significance of the potential neurotoxic effects associated with this type of pesticide?

Question 10. *What changes or additions to the document would improve its readability and make it easier for general audiences to understand?*

In order to organize the responses to these questions, the ten specific questions have been combined into six somewhat broader topic areas:

- ❏ General weight-of-the-evidence issues related to the use of blood and brain measures as critical effects, differences between plasma and RBC measures and their use, and the weight-of-the-evidence approach (*Questions 1, 2 and 9*);
- ❏ Peripheral nervous system measures (*Questions 3 and* 4);
- ❏ Comparative measures in the young and adults (*Questions 5 and 6*);
- ❏ Additional neurochemical measures (*Questions 7 and 8*);
- ❏ Other comments.
- ❏ Editorial comments on the science policy paper (*Question 10*).

B. SUMMARY OF SIGNIFICANT REVISIONS TO THE 1997 SCIENCE POLICY

In the past, OPP generally used the lowest NO(A)EL from among those for plasma, RBC or brain cholinesterase inhibition, or cholinergic effects as the critical effect(s) when deriving reference values such as reference doses (RfDs) or reference concentrations (RfCs). Opinions among commenters diverged greatly concerning the interpretation and use of blood measures of ChE inhibition, particularly plasma cholinesterase for this purpose. Some commenters recommended that little or no reliance be placed on measures of cholinesterase inhibition in plasma and/or little reliance on red blood cell measures of acetylcholinesterase as critical effects. Others offered support for OPP's traditional practice.

The weight-of-the-evidence approach that OPP proposed to use when evaluating the potential of a pesticide to induce cholinergic effects of concern was first described in the 1997 policy document and is now more fully described in the revised 2000 policy. The revised policy still places an emphasis both on findings of functional effects and measures of cholinesterase inhibition when weighing the evidence for selecting an endpoint(s) for quantitative risk assessment (*i.e.*, reference dose or reference concentration derivation). The revised policy explains, in greater detail than did the 1997 policy, that this emphasis is based on the accepted mechanism of toxicity for induction of adverse cholinergic effects on nervous system function (*i.e.*, the inhibition of acetylcholinesterase as a key event) and on the existing limitations in evaluations of behavioral or physiological changes (*i.e.*, functional data). The revised policy also expresses a preference for, and places importance on, direct measures of acetylcholinesterase inhibition in the nervous system. As did the 1997 policy, the revised policy indicates that measures of blood cholinesterase (*i.e.*, measures in non-neural tissues) can provide important insights into potential hazard. The revised policy clarifies that the use of blood measures as surrogates for the nervous system is a matter of prudent science policy in protecting human health, given the existing limitations in evaluations of functional effects, and given that measures of cholinesterase inhibition in brain and peripheral neural tissues are not available in

humans and that peripheral nervous system tissue data are rarely available in animals.

In response to comments, the revised policy acknowledges that inhibition of cholinesterase in the blood is not an adverse effect in itself, but may indicate the potential of an anticholinesterase pesticide to inhibit acetylcholinesterase (the target enzyme) in the nervous system, which, in turn, may lead to adverse effects on nervous system function. Also the revised policy articulates a preference, generally, for the use of reliable red blood cell data (*i.e.,* measures of acetylcholinesterase inhibition) over plasma data (which, in non-human test species, contains a mixture of butyrylcholinesterase and acetylcholinesterase), but also describes situations where plasma cholinesterase data may be preferred.

In response to certain of the recommendations of the 1997 FIFRA Scientific Advisory Panel (SAP, 1997) and a 1997 expert panel of the International Life Sciences Institute (Mileson, et al., 1999), OPP articulates its commitment to supporting the development and validation of methodologies for measuring peripheral nervous tissue acetylcholinesterase activity. As peripheral nervous system data are received, and if valid, these peripheral data can then be used as an alternative or complement to the blood measures (i.e., as a surrogate for the peripheral nervous system in animals) as the basis for quantitative risk assessment. Even so, blood measures will continue to serve an important role in making animal to human comparisons.

C. COMMENTS AND RESPONSES

1. GENERAL WEIGHT-OF-THE-EVIDENCE ISSUES

Question 1.	*How should OPP use measures of ChE inhibition in plasma, red blood cells, and brain in determination of critical effect levels and setting reference doses?*
Question 2.	*Should plasma and red blood cell measures of cholinesterase inhibition be treated differently from brain measures of acetycholinesterase inhibition and/or from one another?*
Question 9.	*OPP has proposed to use a weight-of-the-evidence approach that obligates the risk assessor to consider all of the study results as a whole, rather than focusing on any single result in isolation of the others. Is this approach a reasonable means for evaluating the overall significance of the potential neurotoxic effects associated with this type*

These three questions describe the central features of the science policy on the use of cholinesterase inhibition data in risk assessment. *Question 9* addresses the overall approach in terms of the evaluation of the database in relation to selection of endpoints. *Questions 1 and 2* focus on the uses of cholinesterase inhibition measures in brain, peripheral neural tissue, and blood, and whether they should be weighed in the same manner or differently from one another in the risk assessment process. Because the above questions are interrelated, comments on each question are difficult to respond to without considerable repetition. Accordingly, public submissions addressing these questions will be dealt with together in this section.

The issue which received the greatest number and widest range of comments was how OPP applies a weight-of-the-evidence approach that considers all of the data culminating in the selection of a single critical effect to represent the whole database for the purpose of deriving the reference dose or reference concentration for a particular route and duration of exposure. Although most commenters agreed with the general concept of using a weight-of-the-evidence approach, several commenters

thought OPP's description of its approach was too vague. There were also different views on the decision guidance presented for determining how to select an endpoint, a process which is influenced by a number of factors including the completeness of the database, the relative weight placed on more direct measures of cholinergic effects versus surrogate data (*i.e.*, blood cholinesterase inhibition data) for predicting nervous system effects, and what is the most appropriate or relevant critical effect. Several commenters expressed concern that OPP's approach would simply result in selection of the most sensitive effect. The concern also was expressed by some commenters that OPP's weight-of-the-evidence approach, which retained consideration of blood measures of cholinesterase inhibition, particularly in plasma, was in contrast to the approaches recommended by some members of the international community and some other governmental bodies (*e.g.*, World Health Organization/International Programme on Chemical Safety, Canadian Pest Management Regulatory Agency, the California Department of Pesticide Regulation). The pesticide registrants and users recommended alternative approaches to OPP's weight-of-the-evidence approach that would place much less emphasis on blood measures of cholinesterase inhibition. Other commenters (*e.g.*, Wallace Institute, Natural Resources Defense Council, the Farmworker Justice Fund) supported OPP's use of a weight-of-the-evidence approach which retained the potential for use of plasma cholinesterase inhibition and RBC acetylcholinesterase inhibition as critical effects. The general policies and comments on the use of cholinesterase inhibition data are summarized below by organization.

- **World Health Organization/IPCS report and the Canadian Pest Management Regulatory Agency**

Comments:

The World Health Organization (WHO)/International Programme on Chemical Safety (IPCS) submitted a 1998 draft report from the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) on their interpretation of inhibition of acetylcholinesterase

activity (WHO JMPR, 1999). The draft 1998 JMPR report concluded that "The Meeting considered and essentially reaffirmed its previous policy published in 1990 (WHO, 1990), with some clarifications." The Canadian Pest Management Regulatory Agency (PMRA) endorsed and recommended the approach described in the draft 1998 JMPR report.

The draft JMPR report recommended against using plasma or brain butyrylcholinesterase (BuChE) inhibition measures for setting acceptable daily intakes (ADIs) because it does not consider BuChE inhibition to be an adverse effect. However, because plasma BuChE inhibition is an indicator of absorption of the pesticide, the draft JMPR report considers these data useful for monitoring occupational exposures, and indicates that data on BuChE inhibition should always be reported if there are statistically significant differences between treatment and control group measures of butyrylcholinesterase activity.

The JMPR position stated that clinical signs and brain (AChE) inhibition are the primary endpoints of concern and that RBC AChE inhibition is a surrogate for brain and peripheral nervous system AChE inhibition. RBC AChE inhibition data may be used when both central and peripheral nervous system AChE inhibition measures are unavailable or, for acute exposures, when peripheral nervous system AChE inhibition data are unavailable, and there is more inhibition in RBCs than in the brain.

For brain and RBC AChE inhibition, the JMPR concluded that statistically significant inhibition of 20% or more represents a clear toxicological effect. Less than 20% inhibition or non-statistically significant changes of greater than 20% should lead to more detailed analysis, with the toxicological significance of such data determined on a case-by-case basis.

Agency Response:

OPP believes that the weight-of-the-evidence approach described in the revised policy is similar in some respects to the draft 1998 JMPR report's proposed practices with respect to the use of BuChE inhibition data. When selecting an endpoint(s) for derivation of an RfD or RfC, OPP still considers plasma cholinesterase inhibition to be an important observation to consider, but it has indicated a preference for the use of measures of acetylcholinesterase inhibition in red blood cells generally. OPP further agrees with the draft JMPR report on the usefulness of butyrylcholinesterase inhibition data for monitoring occupational exposures. The revised policy describes the circumstances under which OPP would consider use of these data in preference to those on AChE inhibition in RBCs.

As explained later in *Section C-5*, OPP does not agree with the concept of applying a fixed percentage of enzyme inhibition *(e.g*., 20%) to the interpretation of the significance of the level of cholinesterase inhibition.

On a number of other issues, OPP agrees with many of the views and concerns expressed by the JMPR: concerns about the methodological and reporting limitations of older data on both brain and blood measurements; the relative insensitivity of the assessment of clinical signs in animals and humans in many studies; and the difficulty in determining whether clinical effects are centrally or peripherally mediated, and, thus, whether central or peripheral nervous system acetylcholinesterase is the appropriate neural substrate to correlate with a clinical effect.

❑ **California Department of Pesticide Regulation**

Comments:

The California Department of Pesticide Regulation (DPR) presented a document for the 1997 SAP review on their use of cholinesterase inhibition data in risk

assessments for pesticides. As a regulatory endpoint, the California DPR considers use of blood (RBC or plasma) cholinesterase inhibition in humans in the absence of observed clinical signs, given that neither brain nor peripheral tissue AChE is measured in humans. In evaluating animal data, the California DPR uses plasma or RBC cholinesterase data as a regulatory endpoint only under special circumstances, such as in the absence of central or peripheral nervous system AChE inhibition data or when the chemical "does not readily penetrate the blood brain barrier."

In all of these situations, the California DPR considers use of either RBC or plasma cholinesterase inhibition data based on which correlates better with clinical signs or brain AChE inhibition in other studies. Otherwise, California DPR uses RBC as the default blood compartment, since RBCs contain only AChE, the neural form of the target enzyme.

The California DPR has concluded that any statistically significant inhibition of brain AChE (rather than a fixed minimum percentage) will probably cause some deleterious effect, but that clinical signs at higher doses may be considered to interpret brain enzyme inhibition at lower doses. California DPR similarly concluded that any statistically significant acetylcholinesterase inhibition in peripheral neural tissue also generally is considered to be an adverse effect.

Agency Response:

OPP agrees that the level of inhibition in brain AChE at which clinical signs are seen varies widely among the organophosphorus pesticides and agrees with California DPR's conclusion that any statistically significant inhibition (rather than a fixed percentage like 20%) probably reflects some adverse effect. OPP also agrees that any statistically significant inhibition of AChE in peripheral neural tissue should be regarded as an adverse effect.

OPP also generally agrees with the California DPR's approach to identifying

situations in which RBC or plasma cholinesterase inhibition might serve as the default regulatory endpoint: where central and peripheral nervous system AChE was not or could not be measured, and where no clinical signs were observed. The California DPR position is to choose the blood measures better correlated with clinical signs or with brain AChE inhibition. OPP agrees that this is a valid approach where data are available to make such comparisons. It should be noted, however, that it may be difficult to make such analyses because brain AChE inhibition measures are taken infrequently or never (in the case of humans) and dose effect data are often quite limited. When comparing blood measures of cholinesterase inhibition to central nervous system measures of AChE inhibition, it should be recognized that the comparison is made across two different compartments which will have different pharmacokinetic and pharmacodynamic properties in response to chemical exposure. Thus, there may be differences in the dose delivered to each compartment and the characterization of potency may be confounded.

The California DPR policy also expresses a preference for reliance on blood cholinesterase inhibition (as a peripheral nervous system surrogate) when there is limited penetration of the chemical into the central nervous system. OPP believes that this is a plausible concept but somewhat impractical in that it would lead to the need for an estimate of the penetration of the central nervous system by the pesticide and for establishing decision guidance about how little central nervous system penetration would justify reliance on the blood and whether reliance would be absolute or graded. In any case, there are examples of cholinesterase-inhibiting substances that never enter the CNS, but nonetheless elicit significant toxic effects, including death. In OPP's view, it is better to consider the peripheral nervous system separately from the central nervous system, given the likely pharmacokinetic and pharmacodynamic differences between these two nervous system compartments. OPP views the blood as an appropriate surrogate for the peripheral nervous system even when central nervous system measures are available, and places the burden of proof on those who wish to argue for use of the central nervous system measure as a peripheral nervous system surrogate to provide the appropriate comparative pesticide data on cholinesterase

inhibition in these different compartments. The IWG also raises this issue without proposing how to estimate this parameter, asserting generically that organophosphorus pesticides do penetrate the central nervous system. This issue is discussed further in *Section C-6*.

The California DPR, after the considerations noted, default to RBC AChE data when using blood measures. OPP views the weight-of-the-evidence approach described in its revised policy paper to be generally consistent with the California DPR approach. OPP indicates a preference, generally, for AChE inhibition in RBCs, if certain criteria are met (e.g., the data are reliable) over plasma cholinesterase inhibition, including in certain circumstances where the plasma data show a lower NOAEL. OPP further indicates a preference for measures of peripheral neural AChE inhibition data to move beyond reliance on the blood measures for use in the identification of points of departure for RfD/RfC derivation.

❑ **FQPA Implementation Working Group**

Comments:

The FQPA Implementation Working Group (IWG) submitted an issue paper, as well as extensive comments and answers to the questions posed by EPA. The IWG addressed a wide range of issues, including cumulative risk assessment and the FQPA safety factor. The IWG stated that EPA has not provided a substantial explanation of its science policy concerning cholinesterase inhibition. The IWG asked that EPA acknowledge that inhibition of blood ChE is not adverse in itself and that use of blood measures "has the effect of adding a safety factor." The IWG expressed concern about the use of blood measures which are not adverse in themselves and their associated no effect levels in contrast to using measures of other biological changes that the IWG regarded as adverse effects and their NOAELs as appropriate endpoints for the derivation of reference doses.

The IWG specifically proposed a hierarchy for selection of endpoints, with preference, in order from higher to lower: the NOAEL for signs and symptoms in humans; the NOEL for red blood cell AChE inhibition in humans, if good sign and symptom data are absent; the NOAELs from clinical signs, other neurotoxic effects, and brain AChE inhibition from animals; the NOELs for RBC AChE inhibition, but only in the absence of these other, preferred data. However, if plasma cholinesterase inhibition in animals correlates better with adverse effects than does enzyme inhibition in RBCs, then it may be used.

Agency Response:

The issues concerning cumulative risk assessment and the FQPA 10X safety factor are beyond the scope of the OPP "Science Policy on the Use of Data on Cholinesterase Inhibition for Risk Assessments of Organophosphorus and Carbamate Pesticides" and thus will not be addressed here. OPP currently is developing a guidance document for cumulative risk assessment, early versions of which were presented to the SAP for their review in September and December 1999 (US EPA, 1999b, 1999d). OPP also has prepared a policy paper on the determination of the appropriate FQPA Safety Factors for use in the tolerance-setting process (US EPA, 1999a). This document also has been presented to the SAP and the general public for comment. Thus, OPP will consider any comments from the public received during the comment period on the cholinesterase policy on cumulative risk and the FQPA 10X Safety Factor when responding to comments on the documents prepared specifically on cumulative risk and the FQPA Safety Factor. OPP currently is summarizing and evaluating the comments received on the FQPA Safety Factor paper. It is anticipated that OPP will issue the Cumulative Risk Guidance document for 60-day public review and comment soon.

In response to the comments received that are specific to the cholinesterase policy, OPP has revised the 1997 policy paper to provide a more substantial and detailed explanation of the scientific basis for its science policy (US EPA, 2000). In the

revised policy paper, OPP has expanded its explanation of the rationale underlying the weight-of-the-evidence approach. OPP also acknowledges in this revised paper that cholinesterase inhibition in blood is not adverse in itself. OPP still believes that use of blood measures is justified under certain circumstances, e.g., when OPP lacks animal data on AChE inhibition in peripheral neural tissues, or in the absence of central or peripheral nervous system AChE inhibition measures in humans, even though functional data on behavioral or physiological effects may be available. Also see the OPP response in *Section C-3* for additional discussion on this topic.

OPP disagrees with the proposal that a rigid hierarchical approach be used as recommended by the IWG. As discussed in the revised policy paper, the scope of cholinergic effects is potentially very broad, given that there is cholinergic innervation in virtually every organ in the body. However, existing evaluations of potential behavioral and physiological changes caused by anticholinesterase pesticides generally are quite limited in their scope and extent of assessment. Thus, functional data cannot be relied on solely, to the exclusion of other important kinds of information, in selecting endpoints for the purpose of calculating an RfD or RfC. Given that inhibition of acetycholinesterase is a key event in the mechanism of toxicity that leads to cholinergic effects, measures of cholinesterase inhibition should be considered in the risk assessment process. Although OPP indicates in its policy a preference for having neural measures of AChE inhibition as data points when weighing the evidence, peripheral nervous system data are rarely available. OPP acknowledges that the blood data are surrogate measures of cholinesterase inhibition in the neural tissues of interest, and often represent the only information available to evaluate potential effects in the peripheral compartment, where many effects of concern are elaborated. Cholinesterase inhibition measures in the blood also are viewed as a reasonable surrogate for the peripheral nervous system given that blood is the pharmacokinetic compartment into which chemicals are absorbed and transported to the peripheral nervous system. OPP indicates in its revised policy paper that red blood cell measures of acetylcholinesterase inhibition, if reliable, generally are preferred over plasma data. Since the red cell contains only acetylcholinesterase, the potential of a chemical to

exert effects on neural or neuroeffector acetylcholinesterase may be better reflected by changes in red blood cell acetylcholinesterase than by changes in plasma cholinesterases which contain both butyrylcholinesterase and acetylcholinesterase in varying ratios depending upon the species. This conclusion rests on data showing that a chemical may have significantly differential affinities for binding with AChE and BuChE, among other characteristics.

OPP believes that consideration of functional data, cholinesterase inhibition data in brain, peripheral nervous system, plasma, and RBCs in a weight-of-the-evidence analysis, where all information is considered in an integrative manner, reflects the exercise of informed scientific judgment and also represents prudent health protective policy. Consistent with this approach is the use of blood cholinesterase inhibition (plasma or RBC) in monitoring occupational exposures, where workers are removed from the workplace even if complaints or symptoms indicative of cholinergic effects are not manifested.

Lastly, the issue of the use of the central nervous system cholinesterase inhibition data in preference to measures in blood as a surrogate for peripheral nervous system measures of AChE inhibition is discussed in *Section C-6.* The issue of uncertainty factors is discussed in *Section C-2.*

❑ **Alliance for Reasonable Regulation of Pesticides**

Comments:

The Alliance for Reasonable Regulation of Pesticides (ARRP), under cover of a letter from the American Crop Protection Association (ACPA), submitted a detailed analysis elaborating on the comments of the IWG, and summarizing the comments and analyses of the Acute Cholinesterase Risk Assessment (ACRA) Workgroup. The ACRA made a presentation at the 1997 SAP meeting, providing an overview of its peer reviewed expert report and offering an alternative policy proposal. In addition to echoing many of the comments raised by the IWG, the ARRP also advocated reducing

the default composite intra- and inter-species uncertainty factor of 100-fold to 10-fold when NO(A)ELs for RBC AChE data are used from animal studies as the basis for deriving RfDs/RfCs. The ACRA Workgroup further indicated that peripheral nervous system AChE inhibition measures are unnecessary on the grounds that RBC or brain AChE measures are more sensitive measures. These comments on peripheral nervous system AChE inhibition measures are addressed below in *Section C-6*.

2. USE OF A 10-FOLD REDUCTION IN THE DEFAULT COMPOSITE INTRA- AND INTER-SPECIES UNCERTAINTY FACTOR WHEN THE CRITICAL EFFECT IS BLOOD CHOLINESTERASE INHIBITION

The American Crop Protection Association (ACPA) argued that when blood measures (*i.e.*, plasma or RBC cholinesterase inhibition data) from animal studies are used as the critical effect, "based on a lack of adversity," a composite uncertainty factor of 10-fold should be used instead of the 100-fold factor, when animal data are used as the basis for deriving an RfD or RfC. In typical risk assessments, as a default, the NOAEL for the critical effect is divided by an uncertainty factor of 10-fold for intraspecies and another 10-fold for interspecies extrapolation.

Agency Response:

OPP acknowledges that there could be some conservatism in an assessment when using blood measures of cholinesterase inhibition as a surrogate for enzyme changes in neural tissues. The opposite might well be true, also. Given that there are very few comparative data on cholinesterase inhibition in the peripheral nervous system, brain, RBCs, and plasma, it is not well understood how conservative, if at all, the use of blood data may be compared to the use of the neural tissue data. So, how much the 100-fold factor could be reduced as a general practice cannot be established with any degree of confidence. The better approach would be to collect the actual AChE inhibition measures in the peripheral nervous system and do a chemical-specific analysis as endorsed by OPP's policy paper.

Further, the 1997 SAP supported the use of the traditional uncertainty factors for inter- and intraspecies extrapolation. In discussing species differences, the SAP identified several factors that vary between species and noted that the inhibitory potency of some anti-cholinesterases is species dependent and may be accounted for, in part, by the fact that species vary in their concentrations of blood borne or hepatic enzymes which may activate or degrade certain pesticides. With respect to

intraspecies variation, the SAP noted genetic differences, drug interactions, and nicotine addiction in smokers as some of the factors which contribute to variations among humans. The SAP offered the opinion that both the differences among humans and the differences between humans and test animals that could affect susceptibility to the adverse effects of these chemicals are still poorly understood and, in light of these facts, retention of both standard 10-fold uncertainty factors, as the default, is warranted. OPP agrees with this position.

3. POINT OF DEPARTURE FOR DERIVING REFERENCE DOSES: NOELs OR NOAELs

The point of departure (PoD) is generally defined as a point estimate of an empirically-measured or modeled dose or exposure level that is used as the "jumping-off" point for extrapolation to exposure levels below those tested, where actual human exposures are likely to be occurring. In the case of assessments of effects known or assumed to have a non-linear dose response, the PoD is used to calculate reference values such as a reference dose (RfD) or concentration (RfC). The PoD can be a dose at which no effects are found or a dose level which is associated with some percent of response relative to the control or baseline level of response.

Comments:

Several commenters discussed the issue concerning the use of no-effect-levels (NOELs) or no-adverse-effect-levels (NOAELs) as points of departure when calculating reference doses or concentrations, as well as the terminology that should be used to describe these points of departure. The IWG stated, "The Agency should rely on no-observable-adverse-effect level (NOAEL) data rather than no-observable-effect level (NOEL) data in making risk assessment decisions. If NOAEL data do not exist, the Agency should compel its production. Any reliance on NOEL data should only be on an interim basis, pending the development of NOAEL data."

The Natural Resources Defense Council stated, "Using the terminology of risk assessment, the FQPA requires the basing of reference doses and tolerances on true NOELs (no-observed-effect-levels), in essence, the dose level just below the lowest one observed to cause an effect, and not on NOAELs or no-observed-adverse-effect levels. The mandate for using true NOELs was made explicit in the legislative history of the Food Quality Protection Act by the National Research Council."

Agency Response:

OPP does not believe that the FQPA mandates that EPA, in evaluating animal studies, use a NOEL instead of a NOAEL. The statute does not mention either term. The legislative history does at one point use the term NOEL but that legislative history does not indicate that Congress intentionally used the term NOEL because it did not think it appropriate for EPA to consider the NOAEL. H. Rept. 104-669, 104th Cong., 2d Sess. 41 (1996). In fact, Congress appears to have assumed NOELs are NOAELs. For example, in defining "threshold effect" Congress stated that this "is an effect for which the Administrator is able to identify a level at which the pesticide chemical residue will not cause or contribute to any known or anticipated harm to human health. Id. (emphasis added). If Congress had intended that threshold effects be based on NOELs rather than NOAELs, it would not have used the word "harm" in defining the effect.

Congress seems to have used the term NOEL because it was common usage for OPP at that time. Prior to 1998, in OPP's discussion of the hazard identification process of evaluating pesticide toxicity including cholinesterase inhibition, the term NOEL was used to describe the dose level at which no significant adverse effects were noted. OPP's terminology, however, was not consistent with the rest of the Agency as illustrated in EPA's Integrated Risk Information System (IRIS). This system included more hazard terms than OPP generally employed, including NOAEL, LOAEL and FEL (Frank Effect Level). On September 2, 1998, this apparent semantic inconsistency was eliminated by HED SOP 98.3 which indicated that OPP would use the terms NOAEL

and LOAEL in their scientific reviews and documents. It also stated, “In a practical sense, the terms NOEL and NOAEL have been used interchangeably in OPP. As a general rule, OPP would consider as appropriate for hazard identification and risk assessment only those effects which are adverse or potentially adverse. This inclusion of the term NOAEL should not change any of our hazard endpoints for regulation but add to the quality of our risk assessment.”

4. HUMAN STUDIES

OPP noted in its 1997 policy paper that in the selection of a critical effect, "Valid and reliable human data, when available, take precedence. Because human health is our focus, if there are human data available, it eliminates uncertainties related to inter-species extrapolation and makes the prediction of potential human health effects more direct than if only data on animals are used.”

Comments:

Several commenters, including California DPR, IWG, ARRP, and DOW Agrosciences supported the use of human data over animal data, if appropriate and reliable for use in elucidating the relationship between animal and human responses and the selection of appropriate uncertainty factors and when making risk assessment decisions. Canadian PMRA asked for greater discussion of this issue with respect to the appropriate use of human data for assessing acute and chronic risks, and the recent concerns about the ethical considerations about their use. The Farmworker Justice Fund recommended that any human data be based on those actually exposed in real world conditions-- mixers, loaders, and applicators of pesticides, and harvesters of treated crops-- rather than on human laboratory studies, which they find contrived and more difficult than are monitoring studies to extrapolate to real world situations.

Agency Response:

Initially, it should be noted that OPP neither requires nor encourages the conduct of human hazard identification studies under the Food Quality Protection Act [FQPA]. EPA currently is reviewing its policy concerning the use of human studies of various kinds with respect to ethical and scientific standards for their acceptability and use in risk assessments and regulatory decision making, particularly with respect to decisions under FQPA. EPA has held two meetings of a joint SAP/SAB Panel (December 1998 and November, 1999 (US EPA, 1998, 1999c) on the ethical elements of this issue. The Panel's report will contribute to the articulation of this aspect of a policy related to use of these studies.

OPP agrees with commenters that there may be value to the use of human data in the risk assessment process. In the revised policy paper, OPP has expanded the discussion on the strengths and limitations of human hazard identification studies. Also, the revised policy paper retains the essence of the statement in the 1997 policy when saying that " If scientifically valid, reliable, and ethically appropriate to use, human data may be preferable to animal data because they preclude the need for extrapolation of results across species, thereby avoiding the uncertainties inherent in this aspect of the risk assessment process." However, at this time, OPP's policy is not to use NO(A)ELs identified in human hazard identification studies as the Point(s) of Departure in the derivation of RfDs/RfCs, until such time as an Office policy on the use of human data has been developed. Development of this policy awaits receipt of the SAB/SAP report.

OPP also agrees with the observation by Canadian PMRA that most of the human hazard identification studies have involved acute exposures, or exposures lasting from a few weeks to a few months, and that when assessing risks related to chronic exposures, data from studies of these shorter durations do not seem useful unless it can be shown by empirical data on time-to-peak effect, time-to-steady state for the response and other information from these shorter term exposures that one could

accurately predict the risks of chronic exposure.

OPP considers exposure monitoring studies to be an important part of the overall evaluation of potential exposure and hazards in making risk assessment decisions about pesticide risks.

5. 20% BLOOD OR BRAIN CHOLINESTERASE INHIBITION AS A MINIMUM SIGNIFICANT EFFECT

Comment:

Some reviewers (*e.g.*, IWG, ACPA, DOW Agrosciences) recommended the use of a fixed percentage of cholinesterase inhibition in blood or in brain of 20% for making judgments about the biological and toxicological significance of the effects of all cholinesterase inhibitors, asserting that only if the percentage of inhibition exceeded that value would it represent an effect of concern.

Agency Response:

OPP states in both the 1997 and the revised policy paper that statistical significance should be the primary, although not exclusive, determinant of toxicological significance. The judgment with regard to the biological significance of any changes noted must also be made in parallel with the statistical analysis. This view of statistical and biological significance is consistent with Agency-wide practice for most toxicity endpoints. This approach treats cholinesterase activity data like most continuous endpoints (*i.e.,* graded measures of response such as changes in organ weight, hormone levels or enzyme activity), where no fixed generic percentage of change from the baseline is considered to separate adverse from non-adverse effects (US EPA, 1995; 1996). The proposal for a fixed percentage for both blood and brain inhibition and for all cholinesterase inhibitors is too broad, and should not be generalized. OPP believes that a fixed percentage for describing adversity that would apply to all

organophosphorus pesticides or to all compartments (*i.e.*, blood, central nervous system, peripheral nervous system) cannot realistically be determined or scientifically justified. Each data set must be judged on its own merits, consistent with the weight-of-the-evidence approach that OPP is implementing. Experimental group size, the adequacy and accuracy of the measurement tools and whether or not the treated subjects serve as their own control (i.e. measures are taken before and after treatment in the same individual, then statistically analyzed as such) or a separate control group is used to compare responses of the treated subjects with those untreated subjects are but three of the factors that must be considered when reaching judgments about the biological significance of the observed results. In general, OPP's many years of experience with the review of toxicity studies with cholinesterase-inhibiting substances show that differences between pre- and post-exposure of 20% or more in enzyme levels is nearly always statistically significant and would generally be viewed as biologically significant. The biological significance of statistically-significant changes of less than 20% would have to be judged on a case-by-case basis, noting, in particular, the pattern of changes in the enzyme levels and the presence or absence of accompanying clinical signs and/or symptoms.

California DPR noted that, for at least some agents, overt clinical effects may be seen at levels of acetylcholinesterase inhibition in the brain of less than 20%. Canadian PMRA noted that "there is no clear indication of what level of brain cholinesterase inhibition is tolerated and that subtle centrally mediated effects may not be detected."

The variability of cholinesterase activity measures in some tissues or studies may provide for detection of changes of less than 20%. Mileson *et al.* (1999) found coefficients of variation (mean and standard deviation) between 10% and 20% for serum, brain, and a number of peripheral neural tissues, so that statistically significant differences could be identified in those tissues at less than 20%.

The SAP also expressed another concern about 20% as a threshold level of

inhibition, stating that, "This cutoff value seems reasonable on the surface, but when dose-response curves are steep, it could lead to RfDs uncomfortably close to those that actually cause toxicity."

The WHO argued that with statistically significant cholinesterase inhibition of 20% or above, there is presumed to be a clear toxic effect (WHO, 1990). OPP agrees. With statistically significant cholinesterase changes of less than 20% or greater than 20% without statistical significance, WHO argued that more detailed analysis is needed. OPP agrees here as well, given that OPP evaluates all studies and pesticides on a case-by-case basis.

In conclusion, as for most continuous or graded measures of biological response, OPP thinks that judgments should be made on a case-by-case basis with regard to each study that consider the key factors related to the biological significance of the response, the size and statistical analyses of the study, and the nature and conduct of the assay. To select a standard default percentage of enzyme inhibition to apply in all cases could lead, in our view, to disregarding important information and might, therefore, represent the application of poor scientific judgment.

6. PERIPHERAL NERVOUS SYSTEM MEASUREMENTS OF CHOLINESTERASE INHIBITION

Question 3.	*How should measures of [acetylcholinesterase inhibition in] peripheral tissues be used in these processes of risk assessment, both in a practical sense and a science policy sense?*
Question 4.	*Can measures of acetylcholinesterase inhibition in peripheral tissues, such as the heart and salivary glands, be used as a supplement or even an alternative to blood measures?*

OPP indicated in the 1997 policy paper that measurements of acetylcholinesterase activity in peripheral nervous tissue in animals could provide a scientific means for resolving the longstanding debate about whether it is appropriate to

use blood measures of cholinesterase inhibition to set reference doses and reference concentrations. If data on the peripheral nervous system were available, they could provide a means to refine the risk assessments for anticholinesterase pesticides. A number of questions were posed to the 1997 SAP on the utility, feasibility, and conduct of such testing.

The 1997 SAP responded favorably to this idea (SAP, 1997). The Panel's report noted that "it is important that joint efforts be mounted to evaluate AChE inhibition in the peripheral nervous system *per se* and in the neuroeffector junctions." The SAP further indicated that peripheral nervous system measures "would be extremely important in establishing the value of blood cholinesterase information in predicting peripheral effects." The SAP expressed the view that it is technically feasible at this time to routinely conduct cholinesterase assays in the peripheral nervous system, while also recognizing some of the difficulties involved. The SAP suggested skeletal muscles, heart, lung, salivary glands, diaphragm, and autonomic ganglia (*e.g.*, superior cervical ganglia) as appropriate tissues to consider examining. The SAP considered standardized and reproducible dissection and homogenization of tissue, assays with minimal tissue dilution, selection of the most relevant tissue targets, and standardization of tissue storage conditions to be the most important technical issues.

As a follow up to these recommendations, OPP, under its cooperative agreement with the International Life Sciences Institute (ILSI) Risk Science Institute, requested that the Institute convene a workgroup to develop guidance on the design and interpretation of studies of cholinesterase inhibition in the peripheral nervous system. This group's report, included in the docket for this review, provides further support for the utility and feasibility of this type of study, as well as some additional studies, and recommendations for further research (Mileson, et al., 1999). The ILSI report concluded that "methods and techniques currently available are adequate to characterize the AChE activity in the peripheral nervous system, but additional studies would help to improve these methods." (See also, Marshall, et al., 1999).

Comments

The Implementation Working Group (IWG) argued that because most organophosphorus pesticides are absorbed into the central nervous system, measures of central nervous system cholinesterase inhibition are adequate surrogates of inhibition in the peripheral nervous system, and so reliance on blood measures of cholinesterase inhibition as surrogates for peripheral tissues is not needed.

Agency Response:

There are too few comparative data between blood and brain measures of cholinesterase inhibition and measures of AChE inhibition in the peripheral tissues to state that the central nervous system is an adequate surrogate for the peripheral tissues. Thus, at this time, OPP considers cholinesterase inhibition in the blood as a preferable surrogate for the peripheral nervous system compared to cholinesterase inhibition in the central nervous system and clinical signs based upon the state of our knowledge (actually, the lack thereof) and as a matter of science policy in protecting human health. In the revised policy paper, OPP indicates a preference generally for data on RBC AChE, if they are reliable and meet other criteria, over plasma cholinesterase inhibition data. Although the use of blood data (RBC and plasma) is, in part, a matter of a science policy choice, there is also a scientific basis for using blood as a surrogate measure. Like the peripheral nervous system, the blood is also a "peripheral compartment" where chemicals are absorbed and transported to the peripheral nervous system, while the brain is a different compartment with different pharmacokinetic properties. Because evaluations of clinical signs/symptoms or other behavioral/physiological changes are so limited in scope (*i.e.*, the number of effects assessed) and scale of measurement (*i.e.*, present/absent compared to actual quantitative measures), these data should not be relied on solely, to the exclusion of other kinds of data, when selecting an endpoint(s) for calculating an reference dose (or reference concentration). Although most organophosphorus pesticides penetrate the central nervous system, we do not have sufficient comparative data on a representative

number of organophosphorus and carbamate pesticides to generalize that the central nervous system AChE inhibition measures are an adequate surrogate for the peripheral nervous system. A solution to this issue is to measure the level of acetylcholinesterase inhibition in the peripheral nervous system directly.

Comments:

The IWG asked that protocols, guidance, and adequate time to conduct studies be provided for any new data requirements, such as measures of acetylcholinesterase inhibition in peripheral neural tissues. IWG also asserted that EPA needs to assure the registrants that peripheral nervous system data required by EPA would be used instead of the blood measures of cholinesterase inhibition for endpoint selection and RfD/RfC derivation.

ARRP indicated that peripheral nervous system effects already are assessed in the behavioral assessments in the rat neurotoxicity screening studies, that brain and RBC AChE inhibition measures for at least one organophosphorus pesticide are as sensitive or more sensitive than peripheral nervous system measures, and that all of these data are already being collected. Other commenters (*e.g.*, DOW) raised similar concerns, and argued that there is a need for further validation of these methods.

Agency Response:

OPP can provide guidance for any new data requirement as well as adequate time to conduct these new studies. It is incumbent upon the registrants to provide the protocols for Agency review and comment, however, until such time as standard methodologies are available. EPA's National Health and Environmental Effects Laboratory (NHEERL) is working to develop such methodologies. Furthermore, OPP, in its revised policy indicates that if reliable measures of acetylcholinesterase inhibition in the peripheral nervous system are available, they may be used to replace plasma or RBC data for the purpose of endpoint selection for RfD/RfC derivation. Thus, EPA

agrees with IWG that peripheral tissue measures in animals could replace the blood measures for this purpose. The blood measures would continue to be useful, however, in characterizing species differences in sensitivity of response.

While recognizing the potential limitations that currently exist in studies that include measures of acetylcholinesterase activity in the peripheral nervous system, both the SAP and the ILSI workgroup found such measurements to be technically feasible for the routine conduct and characterization of such measures in a variety of peripheral tissues. OPP recognizes that peripheral neural acetylcholinesterase inhibition data have not been widely collected, that standard procedures have not been defined with inter-laboratory validation studies, and that there is limited experience with studies that measure peripheral neural acetylcholinesterase inhibition. OPP states in its revised policy paper that it will continue to support efforts to develop and validate methodologies for measuring peripheral neural AChE activity and collection of such data.

OPP believes, however, that future studies in test animals should continue to include the collection of RBC and plasma measures of cholinesterase inhibition in animal studies. Because blood measures are collected in humans and, given the unavailability of neural tissue AChE measures in humans, animal blood measures provide a means to make animal-to-human comparisons of a pesticide's affect on cholinesterase activity. As OPP gains experience with measures of peripheral neural AChE data, the collection of both RBC and plasma measures of cholinesterase inhibition will also be important in providing some additional means of confirming the results of cholinesterase inhibition within different compartments. Eventually, with adequate information, OPP will be able to address such issues as how many peripheral neural tissues define an adequate set of data for risk assessment purposes.

OPP disagrees with the commenters who argued that peripheral nervous system effects are assessed adequately in the behavioral or physiological evaluations (*i.e.*, in the development of clinical or functional data). OPP believes that there are significant

limitations in these evaluations. As discussed in the revised OPP policy paper, animal and human hazard identification studies are limited in the scope of the evaluations and the scale of the measurements used. Also, human studies are limited in the numbers of subjects, which may affect the power of the study to detect effects of concern. Thus, functional data obtained from human and animal studies should not be relied on solely, to the exclusion of other kinds of data, when weighing the evidence for selection of the critical effect(s) that will be used as the basis of the RfD or RfC.

OPP disagrees with the commenters who argued that other measures, including data on brain or red blood cell AChE inhibition, may be more sensitive than the peripheral nervous system AChE inhibition measures and, thus, peripheral nervous system measures are not needed. AChE inhibition data from peripheral nervous system tissue are more relevant than blood measures of cholinesterase inhibition because they assess peripheral neural tissue directly. Thus, having peripheral measures of AChE would accomplish two purposes: it allows direct observation of the potential for effects in the tissue of concern and it would resolve the debate about using blood measures of cholinesterase inhibition as surrogates for nervous tissue effects in risk assessment. Although blood and/or brain measures of cholinesterase inhibition may be more sensitive than peripheral neural AChE inhibition for some pesticides, the extent to which this can be generalized to all anticholinesterase pesticides cannot be determined at the present time given the paucity of comparative data. At this time, it is premature to conclude that RBC or brain measures are more sensitive than measures in peripheral tissues. A good comparative data set based on cholinesterase inhibition in blood and both nervous system compartments for a number of representative pesticides is the only scientifically sound way of resolving the debate concerning the use of blood data in risk assessment. Because there will always be reliance on blood cholinesterase inhibition measures in humans, the understanding of the relationship between blood cholinesterase inhibition and nervous system AChE inhibition is important for characterizing human risk.

7. METHODS FOR MEASURING CHOLINESTERASE ACTIVITY, INCLUDING

ACETYLCHOLINESTERASE IN BRAIN REGIONS

For several years, EPA has been involved in efforts to standardize methods for measuring cholinesterase activity in experimental animals (US EPA 1992, 1996; Wilson *et al.,* 1996). These efforts were summarized as part of the material prepared for the 1997 SAP review.

Since 1993, neurotoxicity screening studies in the rat conducted on a number of cholinesterase inhibitors as required by OPP have called for assessment of AChE activity in a variety of brain regions, including hippocampus and cerebellum. This was based on the rationale that different brain regions contain varying amounts of AChE, and these regions serve different neurological functions. Thus, a better understanding of the relationship between changes in AChE inhibition in these regions and the functions served by these areas would result from the analysis of AChE inhibition in specific brain regions.

Comments:

One commenter (Compliance Services International) discussed issues related to the assay of cholinesterase activity in toxicity studies submitted to the OPP, and indicated a need for greater standardization of cholinesterase assays (*e.g.,* with respect to substrate, common units, temperature, brain sampling, sample handling and preparation, wave length).

This commenter and a second commenter (DuPont) discussed the variability found in the measurements of AChE in specific brain regions in several studies. DuPont suggested that these data showed the unreliability of such measurements, which rendered them unusable, while Compliance Services International concluded that changes less than 20% would not likely be detectable in those brain regions. Concern also was voiced that reliable dissection was not feasible without specialized training and equipment and that several literature studies failed to find meaningful

differences in brain region AChE inhibition.

Agency Response:

OPP agrees that there are issues related to the standardization of methodology of these assays. While the draft standard operating procedure for cholinesterase assays of organophosphorus pesticides has not been finalized (US EPA, 1996), and no procedure for carbamates is available yet, OPP is aware of these and related concerns identified by EPA's Office of Research and Development, National Health and Environmental Effects Laboratory (see Hunter *et al.*, 1997). OPP emphasizes in the revised policy paper that methodological issues are an important element in the evaluation of cholinesterase data and in the selection of an endpoint(s) for calculation of the reference dose (or concentration). When inadequacies are identified in the methods for measuring cholinesterase activity, or in any other method for measuring endpoint effects, the uncertainties that these deficiencies may raise can be accommodated for in a variety of ways in the risk assessment process.

With respect to measurement of AChE inhibition in different brain regions, OPP believes that standard dissection techniques for rats (e.g., Glowinski and Iversen, 1975) do not require specialized training or equipment and can provide adequate reliability of dissection (with standard deviations of 7-17%). Further, OPP finds that the few studies cited by the commenters comprise too meager a data set from which to conclude that these measures will not show differences for the many pesticides that inhibit cholinesterase.

8. COMPARATIVE MEASURES OF CHOLINESTERASE INHIBITION AND EVALUATION OF FUNCTIONAL EFFECTS IN THE YOUNG AND ADULTS

Question 5.	*Should comparative data on* cholinesterase inhibition *in the young exposed pre-natally, during infancy (nursing), and during childhood be considered essential for defining the relative sensitivity of the young and adults?*
Question 6.	*Are other measures, such as functional measures of clinical signs, or learning and memory, similarly important?*

These two questions were included in the November 5, 1998, notice as a way of seeking guidance as to how the OPP cholinesterase policy should address FQPA concerns regarding the relative sensitivities of young and developing individuals in comparison to mature individuals.

Comments:

Several commenters supported using comparative cholinesterase data in risk assessment on the grounds that cholinesterase enzyme inhibition is often the most sensitive response to a cholinesterase inhibiting chemical. In addition, the blood brain barrier may not be as well developed in young animals and humans as in adults, and, therefore, young individuals may be more or less sensitive than adults. Canadian PMRA notes that "the comparative data on ChE inhibition in the young exposed pre-natally, during infancy or childhood should be considered essential for defining the relative sensitivity of the young and adults." Some commenters (e.g., NRDC) advocated requiring the cholinesterase inhibition data in support of the intent of FQPA to assure that there are adequate and reliable data for assessing the risk to infants and children, and assuring their protection. These commenters stated that, under FQPA, it is incumbent on the Agency to ensure protection of infants and children, which should be achieved by evaluating comparative responses on the basis of the most sensitive indicators. They further argued that cholinesterase inhibition cannot be disregarded in the face of this FQPA mandate to evaluate sensitive endpoints, given that acetylcholinesterase performs a critical role in the mammalian nervous system and is, by design, the target enzyme for a number of pesticides.

Comments offered by the IWG, which several other commenters endorsed, did not support using cholinesterase data in defining relative sensitivity of young and adults, at least until some further research on such potential relative sensitivities has been conducted. The basic reason for not requiring such cholinesterase data at this time is an assertion that these data would not be useful in characterizing age-related sensitivity. The IWG position, which is based on the premise that cholinesterase inhibition is not an adverse effect, states that "As we have already pointed out, measurements of cholinesterase activity would have little or no relevance to determinations of the relative sensitivity of young and adults to adverse effects," and further along: "Relative sensitivity can only be established by measuring and comparing specific adverse effects in whole animals..." Another commenter simply argued that the

effort would be irrelevant because cholinesterase inhibition is not an adverse effect.

Only a few commenters responded to Question 6 regarding the importance of other measures, such as functional measures of clinical signs, or learning and memory testing in the assessment of comparative toxicity in young and adults. Two commenters expressed opposition to such further testing, arguing that effects on the parameters in question would not be seen except at doses eliciting extensive cholinesterase inhibition. However, one of those two commenters did support an enhanced assessment of clinical signs.

Comments by the IWG, which were endorsed by a number of the other commenters, favored additional research in the area before deciding whether to impose such testing. They suggested reviewing the literature. If the available information is not adequate to resolve the concern, basic research should be conducted to determine relative sensitivity/susceptibility of adult and young animals to a few representative compounds at environmentally relevant doses.

Four commenters supported the additional testing, generally via implementation of the developmental neurotoxicity study. A principal reason offered for requiring such data would be to ensure *reasonable certainty of no harm*, as specified by the FQPA. One commenter argued that assessment of effects on learning and/or memory may be equally as essential and important as assessment of cholinesterase inhibition in revealing neurotoxicity. The commenter suggested that such tests would serve to address the emphasis of FQPA upon obtaining complete and reliable data on potential toxicity of infants and children.

Agency Response:

OPP believes that, for cholinesterase-inhibiting chemicals, measures of cholinesterase inhibition and assessment of cholinergic function (which includes learning and memory) are specifically appropriate and important for the evaluation of these classes of chemicals in the assessment of potential hazards to infants and children. This position is consistent with OPP's determination that cholinesterase

inhibition is a valid indicator of potential hazard, by the wealth of data supporting the use of this end point in the assessment of risk, and the growing literature on the effects of early exposures on cholinesterase inhibition and neurobehavioral measures (Environmental Health Perspectives, 1999; a special review supplement, which featured four articles that described the influence of cholinesterases on the development of the nervous system (Bigbee, et al; Brimijoin and Koenigsberger; Lauder, et al.; Slotkin)).

Although cholinesterase measurements are not included currently as endpoints in the guidelines for the prenatal developmental toxicity study or the two-generation reproduction study, they recently have been added to the developmental neurotoxicity study when it is to be conducted with a cholinesterase-inhibiting substance. A number of chemical-specific studies have been received by OPP that have assessed cholinesterase inhibition in fetuses or young animals following developmental exposure or early post-natal exposure. This topic was specifically addressed in a presentation to the FIFRA Scientific Advisory Panel in December, 1998 on a “Retrospective Analysis of Twelve Developmental Neurotoxicity Studies Submitted to OPPTS” (Makris et al., 1998). Cholinesterase data in perinatal animals were found to be useful in assessing the potential for effects following direct or indirect exposure to dams and their offspring, and in providing information on comparative effects and dose response. The SAP, in their response to the Agency presentation, recommended that cholinesterase measurements should routinely be made in developmental neurotoxicity studies on acetylcholinesterase-inhibiting chemicals (SAP, 1999).

OPP issued a data call-in the Fall of 1999 which requires the registrants of all currently-registered organophosphorus pesticides to conduct the acute and subchronic neurotoxicity screening studies in the (adult) rat and the developmental neurotoxicity study in rats. OPP has been working with an ACPA workgroup to reach agreement on adjustments needed to the standard developmental neurotoxicity study design to enhance the value of the results of this study as it relates to the cholinesterase-inhibiting organophosphorus pesticides. Modifications to the standard protocol will

include the addition of measurements of cholinesterase enzyme activity in both the dams and the offspring. Details of this and any other modification will be documented elsewhere.

9. ADDITIONAL NEUROCHEMICAL MEASUREMENTS

Question 7. Should EPA require the differentiation of acetylcholinesterase and butyrylcholinesterase in plasma, and how might this data be used?

Of the two common blood measures, red blood cells contain only acetylcholinesterase, which is identical to the neuronal form of the target enzyme. The composition of plasma cholinesterases varies widely among humans, dogs, and rats, the species in which these measures are most typically made for risk assessment purposes. While human plasma is overwhelmingly BuChE, the plasma cholinesterase of dogs and rats contains both acetylcholinesterase and butyrylcholinesterase. The two plasma cholinesterases may be differentiated by use of selective inhibitors.

OPP currently does not require information distinguishing acetyl- and butyrylcholinesterase from one another and, at present, most studies received by EPA do not differentiate between these enzymes. The 1997 SAP report, however, suggested that differential analyses of butyrylcholinesterase and acetylcholinesterase could serve as a means to provide additional data to refine the assessment of the effects on plasma cholinesterases (SAP, 1997). EPA, therefore, solicited additional comment on this issue.

Comments:

A number of commenters (IWG, DOW Agrosciences, Canadian PMRA) did not support the collection of, or a requirement for the collection of, these data because they generally saw a limited role for blood measures. They believed that measures of inhibition of any blood cholinesterases are of limited significance, assessment of red blood cell activity already measures acetylcholinesterase levels, and the set of data on enzyme activity currently collected is sufficient to conduct risk assessments.

Agency Response:

OPP agrees that because red blood cells of humans and the mammalian species generally used to study cholinesterase inhibition for risk assessment purposes contain the equivalent of the neuronal form of the enzyme acetylcholinesterase and human plasma contains no acetylcholinesterase, there is little value in attempting to differentially determine acetyl- and butyrylcholinesterase activities in human plasma. However, OPP believes that the differentiation of BuChE and AChE may be useful in rat studies, where there is a mix of these enzymes of roughly 50%, and in dog studies, even though the preponderance of plasma cholinesterase in this species is in the acetyl form. OPP is not, at this time, however, proposing to require this type of data, but will revisit the issue in its follow-up strategy to the implementation of the revised policy as it pertains to the evaluation of nervous system function. To facilitate the assessment of function, OPP places a higher priority on obtaining measures of acetylcholinesterase inhibition in neural tissues and to reduce reliance on blood measures in animal studies. Thus, OPP has made a commitment in the revised policy paper to support the development and validation of protocols for measuring acetylcholinesterase inhibition in peripheral tissues.

Question 8. Should EPA require receptor binding assays for long term (subchronic and chronic) studies, and how should such data be interpreted?

Receptor binding assays can quantify the number of cholinergic receptors in specific tissues. Data derived from such an assay could provide valuable information for assessment purposes given that a common consequence of prolonged cholinesterase inhibition in the nervous system is a reduction in the number of cholinergic receptors and this “down regulation” reduces the sensitivity of the nervous system to the continuing stimulation brought about by the inhibition of acetylcholinesterase. This down regulation of cholinergic receptors represents a

longer term response to exposure, the onset of which occurs later than inhibition of acetylcholinesterase. This neuronal down regulation might be differentially affected by certain chemicals, and its time course might differ from that of the acetylcholinesterase inhibition.

OPP currently does not require receptor binding assays and at present, studies received by EPA do not include this information. A suggestion was made in the 1997 SAP report that research on the effects of anticholinesterase chemicals on cholinergic receptor numbers should be pursued (SAP, 1997). EPA, therefore, solicited additional comment on this issue.

Comments:

A variety of views was offered on this issue. Canadian PMRA stated that a requirement of measures of neuronal down regulation of cholinergic receptors would be premature, and that there is no scientific consensus on the interpretation of such data from these assays. Canadian PMRA also suggested that it may be advisable to consider such receptor assays in the future, presumably after their value had been established. The IWG observed that by protecting against cholinesterase inhibition, no down regulation should result. The NRDC observed that receptor binding could reflect chronic effects and be related to what Morgan (1989) described as effects of repeated absorption too low to cause acute poisoning, that is, weakness, malaise, and persistent anorexia. Another commenter (Guillebeau) stated that EPA should facilitate research into receptor binding, but noted that how receptor binding relates to chronic effects should be answered before data interpretation can be properly addressed.

Agency Response:

At this time, OPP is not planning to require receptor binding assays, but OPP encourages further research and discussion on these types of studies. OPP disagrees with IWG, in that OPP finds few data to support the view that acetylcholinesterase

inhibition alone completely describes the potential hazards associated with exposure to pesticides. OPP believes that cholinergic receptor down-regulation in repeated exposure studies is an important issue, and thus OPP will follow this issue closely as research data emerge. Data from receptor binding assays could help to broaden the data base on which to characterize the potential hazard of anticholinesterase chemicals.

10. ADEQUACY OF CURRENT TESTING FOR ASSESSING THE POTENTIAL DIFFERENTIAL TOXICITY OF ENANTIOMERS

Comments:

One commenter (Tweedale) urged EPA to account for enantiomer toxicity in reassessing tolerances for the organophosphorus pesticides. Enantiomers are non-superimposable mirror image molecules produced in the manufacture of organophosphorus active ingredients. For example, two enantiomers are possible for each of the following chemicals: naled, fenamiphos, isofenphos and profenophos. Specifically, the commenter raises concern over the possibility that specific enantiomers of these substances could be produced during manufacture, and that these enantiomers may be more toxic than other enantiomers that may be present. Hence, the risks posed by these substances could be greater than currently perceived.

Agency Response:

EPA believes its current risk assessment approach adequately accommodates for the potential for hazards of enantiomers. OPP recognizes that while enantiomers of a given substance have identical physico-chemical properties (except in the direction in which they rotate a plane of polarized light), they may vary in toxicity and potentially pose different risks to human health or the environment. A given manufacturing process may produce one or more specific enantiomers of the pesticide active ingredient. It is also possible that one enantiomer may be produced more readily than another enantiomer and may predominate in the commercial product. Even if an

enantiomer is formed in a low concentration relative to another enantiomer during synthesis of a commercial product, it may still contribute to the overall risk of the product if its toxicity is significantly greater than the toxicity of the other enantiomer.

OPP routinely evaluates the manufacturing processes used to synthesize a pesticide active ingredient as part of the process to assess the risks posed by pesticides. The primary purpose of evaluating a manufacturing process of a given pesticide is to ascertain the composition of the technical product with regard to overall risk to human health and the environment. This evaluation includes an analysis of the feedstocks, reagents, catalysts, solvents, and any other substances used in the process; reaction conditions; and yield. One purpose of this evaluation is to identify carryover impurities, incomplete reaction products, byproducts, and any other substances that are known or could reasonably be anticipated to be present in the final product produced by the process. OPP also considers any impurities in the reactants or other substances used in the synthesis that may contaminate the technical product and contribute to overall risk. Manufacturers must certify that the composition of the technical product falls within certified limits.

OPP's required toxicity data also provide information on the potential risks of enantiomers. The mammalian toxicity studies submitted by the chemical sponsors are performed with the technical grade of the active ingredient. The results reflect the actual toxicity of the commercially marketed pesticide active ingredient. Therefore, even if one of the two enantiomers of an active ingredient is substantially more toxic than the other, and is present in the test substance, its toxicity would be expressed in the mammalian toxicity data submitted to the Agency and used in OPP's risk assessment of the technical product.

11. EDITORIAL COMMENTS

Question10.	*What changes or additions to the document would improve its readability and make it easier for general audiences to understand?*

Comments:

Several reviewers suggested that some scientific aspects of the paper needed to be clearly explained or explained in more detail, and certain terms needed to be defined. The IWG provided an annotated line-by-line analysis. In addition to the changes in the content of the policy, the IWG suggested changes in the language of the document and its organization.

The Canadian PMRA recommended that the paper make greater distinction, where possible, between butyrylcholinesterases and acetylcholinesterases, as well as between plasma and red blood cell cholinesterases.

Agency Response:

In line with a number of commenters' suggestions, the more detailed discussion of previous SAP meetings and earlier attempts to define a policy have been removed from the document and replaced with a simplified summary. Also, in response to comments, key scientific terms are defined, and, where appropriate, the policy paper now differentiates between acetylcholinesterase and butyrylcholinesterase as well as between plasma and red blood cell cholinesterases. The organization of the document has been simplified. An introductory section has been added that provides some basic discussion of the Agency's risk assessment paradigm as well as an overview of the biology of the cholinergic nervous system and toxicology of acetylcholinesterase inhibition. A brief discussion of the use of cholinesterase inhibition as a regulatory

endpoint in OPP is also included.

The document discusses the different types of data to be considered for risk assessments, i.e., functional cholinergic effects (*i.e.*, clinical signs such as sweating); brain AChE inhibition measures; RBC AChE inhibition measures; and plasma cholinesterase inhibition measures, and the scientific rationale that provides the basis for the weight-of-the-evidence approach to be used in evaluating cholinesterase-inhibiting pesticides. This discussion is followed by the weight-of-the-evidence section, which describes the process for selection of critical effects and includes an analysis of both individual studies and the entire database, the influence of patterns of toxicity (*e.g.,* where cholinesterases in different compartments are differentially affected), and the uses of additional data to refine risk assessments.

D. REFERENCES

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E. LIST OF COMMENTERS

Docket #	OPP-00557 Framework for Addressing Key Issues Presented by (FQPA) as Developed Through the (TRAC)
Paper and Author:	"OPP's Science Policy on the Use of Cholinesterase Inhibition for Risk Assessment of Organophosphate and Carbamate Pesticides" prepared by Dr. William Sette
Comment Number and Date	**sAuthor and Affiliation**
07 - 1/12/98	Jellinek, Schwartz, and Connolly McDermott, Will, and Emery Title of Issue Paper II- Choice and Use of Endpoints in Risk Assessments of Cholinesterase Inhibitors 20 pp.

Docket #	OPP-00560
Paper and Author:	"OPP's Science Policy on the Use of Cholinesterase Inhibition for Risk Assessment of Organophosphate and Carbamate Pesticides" prepared by Dr. William Sette
Comment Number and Date	**Author and Affiliation**
06 - 11/23/98	Roger A. Yeary TruGreen/ChemLawn
07 - 11/27/98	Tony Tweedale Coalition for Health, Environmental and Economic Rights

Comment Number and Date	Author and Affiliation
08 - 12/14/98	IWG (Edward Ruckert of McDermott, Will & Emery)
09 - 12/24/98	Henry Wallace Institute for Alternative Agriculture
10 - 12/29/98	Dr. Paul Guillebeau Pesticide/IPM Coordinator, Dept. of Entomology, U. of Georgia
12 - 12/23/98	Dr. J.L. Herrman WHO Joint Secretary, Int. Programme on Chemical Safety
13 - 1/12/99	Dr. C.A. Franklin, Executive Director, Health Canada
14 - 1/12/99	Dr. Arthur L. Craigmill Extension Toxicology Specialist, UC Davis
15 - 1/12/99	Dr. C.A. Franklin, Executive Director, Health Canada
16 - 1/18/99	Dr. J.R. Tomerlin, Vice President, Novigen Sciences
17 - 1/19/99	IWG
18 - 1/19/99	Dr. John F. McCarthy, Vice President, Science and Reg. Affairs, ACPA
19 - 1/19/99	IWG
20 - 1/19/99	Shelley Davis, Co-Executive Director, Farmworker Justice Fund
21 - 1/19/99	Novartis
22 - 1/19/99	Dr. Daniel M. Byrd III, President, Consultants in Toxicology, Risk Assessment and Product Safety (CTRAPS)

Comment Number and Date	Author and Affiliation
23 - 1/19/99	Dr. David Wallinga, Senior Scientist, NRDC
24 - 1/19/99	W.B. Jenkins, President, North Carolina Farm Bureau Federation
25 - 1/19/99	Nancy Erickson, Director, Illinois Farm Bureau
26 - 1/19/99	Sam Moore, President, Kentucky Farm Bureau Federation
27 - 1/19/99	Raymond R. Casey Vice President - Nat. and Corporate Affairs, Ohio Farm Bureau
28 - 1/19/99	Albert A. Almy, Dir. - Public Policy and Commodity Div., Michigan Farm Bureau
29 - 12/31/98	David J. Clegg, D.J. Clegg Consulting Inc.
30 - 1/19/99	Dan L. Cassidy Director, National Legislative Prog., Missouri Farm Bureau Fed.
31 - 1/19/99	Louie A. Brown, Jr., Director of Food Safety, California Farm Bureau Fed.
32 - 12/17/98	Dr. F.M. Gersich, Leader, Regulatory Success - Americas, Dow AgroSciences
33 - 1/19/99	Dr. Daniel M. Byrd III, President, Consultants in Toxicology, Risk Assessment and Product Safety (CTRAPS)
34 - 1/19/99	Arkansas Farm Bureau Federation
L001 - 1/19/99	Phil Ward, Executive Vice-President, Oregon Farm Bureau